# **Analysis of Resource Allocation in Final Stage Sugarcane Clonal Selection**

J. Steven Brown\* and Barry Glaz

### **ABSTRACT**

Superior genotypes of sugarcane (interspecific hybrids of Saccharum spp.) must continue to be developed with current resources as selection criteria evolve and expand. Developing future cultivars of sugarcane for the Everglades Agricultural Area (EAA) of South Florida with high water table tolerance and increased P-uptake efficiency could be an integral part of Everglades restoration. The objective of this study was to assess the current allocation of resources in the final selection phase of cultivar (clonal) development of the Canal Point, FL, sugarcane breeding program. Variance component analyses were conducted on elite genotypes from 7 yr of trials. Variance components were used to compare relative magnitudes of sources of variation and to explore more efficient use of resources. Variation attributable to  $crop \times location$  interaction was nearly always the largest relative source of variation next to the residual term. The contributions to variance due to genotype  $\times$  crop and genotype  $\times$  location interactions were low, though these interactions cannot be discounted in cultivar release decisions. Variance due to replications was extremely low. Four statistics were used as metrics of experimental precision when reducing the number of replications. Reducing replications from eight to four did not compromise experimental precision. Removing the second-year planting sequence compromised little, if any, useful information for effective cultivar release decisions. Better allocation of resources could be achieved by alternative experimental design scenarios. Testing for high water table tolerance or P-uptake efficiency could also be included, improving ecological compatibility of agriculture in the EAA.

HE SUGARCANE CULTIVAR development program of and University of Florida consists of four clonally propagated selection stages beyond the initial planting of seedlings from true seed. The seedling and Stage I phases are based mostly on visual selection for agronomic type and disease resistance. Stage II is a transition stage in which selection is based on visual assessments and quantitative measurement of sucrose content and sugar yield at one location. Stage III involves testing at four locations with two replications at each location. More extensive quantitative measurement of sugar yield and sugar quality traits occurs at this stage in two-row plots which are 4.5 m long. Approximately 50 000 to 100 000 new potential cultivars are planted annually as seedlings, and 5 yr later approximately 11 of these are selected from Stage III for more extensive testing in Stage IV. Tai and Miller (1989) gave a detailed description of this selection program.

New clones are coded with a "CP" number after selection in Stage I. This naming convention comes from "Canal Point," the location of the breeding program in Florida. Thus, clones with the CP 90 prefix were selected

USDA/ARS, Sugarcane Field Station, HCR Box 8, Canal Point, FL 33438. Received 6 March 2000. \*Corresponding author (jsbrown@saa.ars.usda.gov).

Published in Crop Sci. 41:57-62 (2001).

in 1990 in Stage I, passed successively through Stages II and III, and were first grown in Stage IV trials in 1993. The current procedure for Stage IV, initiated in 1994, is to plant one or more eight-replicate tests at each of nine grower/cooperator farms in plots of three rows 10.7 m long. The outer rows of each genotype are border, and the inside row is used for stalk weight and yield estimates. Prior to 1994, plots consisted of four 10.7-m rows in each of four replications, two rows of which were sampled separately as subsamples. Stage IV trials are harvested annually for 3 yr, as are most of Florida's sugarcane production fields (plant cane, first and second ratoon). These trials are planted over a 2-yr period in two sequences. The majority of the locations are planted in late fall or early winter of the first year, and the remaining locations are planted the following year from late summer through fall. These two sequences fit more appropriately into the planting schedules of the respective cooperative growers.

Limited resources mandate that selection criteria must be carefully chosen. We select primarily for important agronomic characteristics and pest resistance. Although there is limited rotation of other crops with sugarcane in the EAA, sugarcane is essentially a monoculture. Thus, disease pressures are constant and complex due to changes in races of some diseases and arrival of new pathogens. Insect problems, though not ignored, are of lesser importance.

The relationship of sugarcane production to Everglades ecology has recently become a concern in Florida. Kang et al. (1986) discussed the issue of organic soil subsidence, higher water tables, and the need for floodtolerant genotypes. Recent legislation now mandates that the phosphorus discharged to the Everglades in water from Florida sugarcane fields be reduced by at least 25% (Whalen and Whalen, 1994). Up to 16 000 ha of sugarcane may be publicly purchased and used as storm water treatment areas to help meet phosphorus regulations (Stone and Legg, 1992). Growers have implemented "Best Management Practices" to meet goals of phosphorus discharge reduction at an estimated annual cost of \$153 ha<sup>-1</sup> (Stone and Legg, 1992), and are assessed an annual agricultural privilege tax of \$62 ha<sup>-1</sup>. While the Canal Point breeding program must continue to develop clones with superior agronomic performance, it is also possible to assist with Everglades restoration by identifying cultivars that yield well under cyclical floods and have an increased rate of uptake of soil P. 8It has been shown that the range of soil P removal among Stage IV clones is approximately 8.5 kg ha<sup>-1</sup>, indicating that substantial variability among Stage IV clones exists for this trait. (Glaz, 1997a). In addition, cultivar screening has shown significant flood tolerance

**Abbreviations:** CP, Canal Point, FL; EAA, Everglades Agricultural Area; GCV, Genetic Coefficient of Variation.

among commercial sugarcane cultivars (Kang et al., 1986; Deren et al., 1991). With sufficient resources, evaluating clones for flood tolerance and P-uptake ability in Stage IV should alleviate some of the problems associated with soil subsidence and P discharge.

Adaptation to changing needs must be accomplished at the current level of resources. The objective of this investigation was to assess the allocation of resources in Stage IV trials as selection needs evolve over time. Particular attention was paid to numbers of replications, harvests, and planting years (successive plantings) in the Florida CP sugarcane breeding program.

## **MATERIALS AND METHODS**

Variance component analyses were performed for four CP test series to observe relative magnitudes of sources of variation. Estimates of the experiment-wise %CV, genetic repeatability, genetic coefficient of variation (GCV), and  $R^2$  values were obtained. These values were estimated for five metric traits of these trials to assess the appropriateness of resource allocation in these trials.

Variance component analysis was performed with data from four previously tested Stage IV CP-series: CP 89, CP 90, CP 91, and CP 92 (Glaz et al., 1994, 1995, 1997b, 1998). Years and locations in which each series and planting sequence was grown and the number of clones in each trial are given in Table 1. The five traits considered for all analyses were as follows: stalk number per row (16.2 m<sup>2</sup>) for CP 89 and CP 90 or per two-row plot (32.4 m<sup>2</sup>) for CP 91 and CP 92, average stalk weight in kg of 10 sampled stalks per experimental unit, cane yield (Mg ha<sup>-1</sup>), theoretical recoverable sugar (kg sugar Mg<sup>-1</sup> of cane), and sugar yield (Mg ha<sup>-1</sup>). All factors were considered to be random in this analysis. In normal postharvest analyses of these trials, both genotype and crop year (plant cane, first, and second ration) effects and their interaction would be considered as fixed effects; however, the objective of this analysis was to observe relative sources of variation in the testing process, and not to compare and contrast specific genotypes or crops. Proc Mixed of the SAS system was used for the analysis with the Restricted Maximum Likelihood (REML) option (SAS, 1997).

Complete data sets were used for all series except for the second ration crop of the second planting sequence of CP 92, which was not available. The statistical model used for the analyses for all traits of each planting sequence for CP 91 and CP 92 was as follows:

$$Y_{ijkl} = \mu + G_i + C_j + GC_{ij} + L_k + R(L)_{lk}$$
$$+ CL_{ik} + GL_{ik} + GCL_{iik} + \varepsilon_{iikl},$$

where  $Y_{ijkl}$  = observation for Genotype i, in Crop j, in Location k, in Rep l nested within Location k;  $\mu$  = the overall mean;  $G_i$  = the effect of the ith genotype;  $C_j$  = the effect of the jth crop;  $GC_{ij}$  = the interaction of the ith clone with the jth crop;  $L_k$  = the effect of the ith location;  $R(L)_{lk}$  = the effect of the ith replication nested within the kth location;  $GL_{ik}$  = the interaction of the ith clone with the kth location;  $GL_{ijk}$  = the interaction term between the ith clone, the ith crop, and the ith location; and  $g_{ijkl}$  = the residual term.

The same model was used for the CP 89 and CP 90 series with the addition of one term for the effect, Row {Genotype [Replication(Location)]}, to account for the double rows of each plot of each genotype within each replication, and the individual sampling of each row that occurred. The variance components,  $\sigma_G^2$  (broad-sense genetic variance),  $\sigma_{GC}^2$  (interaction of genotype  $\times$  crop),  $\sigma_{GL}^2$  (interaction of genotype  $\times$  location), and  $\sigma_E^2$  (residual error) correspond directly to model effects estimated by Proc Mixed.

Genetic repeatability was estimated as a function of the variance components and the number of genotypes, locations, and replications using the equation:

Genetic repeatability = 
$$\sigma_G^2/(\sigma_G^2 + \sigma_{GC}^2/j + \sigma_{GL}^2/k + \sigma_{GC}^2/jk + \sigma_E^2/jkl)$$
,

where j is the number of crops, k is the number of locations, and l is the number of replications (Milligan, 1994). This calculation for sugarcane, being conceptually close, and computationally identical (Milligan et al., 1990), to broad-sense heritability, estimates the magnitude of genetic variance relative to phenotypic variance. This unitless ratio allows comparisons of genetic expression across traits, time, and in this analysis, across experimental designs. The calculation was the same for the CP 89 and CP 90 series, with the addition of a divisor corresponding to the additional term in the model for two rows within replications. The statistic, GCV (Milligan et al., 1990), was calculated as the square root of broad sense genetic variance estimate ( $\sigma_G$ ) divided by the experimental mean, and expressed as a percentage. This statistic also provides a unitless measure of the genetic variance of a trait relative to its mean, facilitates comparison of traits with different units, and estimates expressed genetic variability when comparing different traits, times, or experimental inputs. Its information content is very similar to genetic repeatability, but presents the information from a different perspective. Experiment-wise %CV was calculated in the usual manner, as the square root of the residual divided by the overall experimental mean  $\times$  100, as an indication of experimental design precision. All models were analyzed again by Proc GLM of SAS to evaluate the  $R^2$  statistic (variance explained by model/total variance) for model fit. Though the  $R^2$  statistic is usually used for variable

Table 1. CP series analyzed, years grown for each series, number of locations, and locations in which trials were grown.

Series	Planting sequence	No. of locations	Years	Locations
CP 89	1	9	1992-1994	Duda, Eastgate, Hilliard, Knight, Lykes, Wedgworth, Okeelanta, Sugar Farms Co-op East, Sugar Farms Co-op West
	2	1	1993-1995	Okeelanta Successive
CP 90	1	7	1993-1995	Duda, Knight, Lykes, Okeelanta, Sugar Farms Co-op East, Sugar Farms Co-op West, Wedgworth
	2	3	1994-1996	Eastgate, Hilliard, Okeelanta Successive
CP 91	1	7	1994-1996	Duda, Knight, Lykes, Okeelanta, Sugar Farms Co-op East, Sugar Farms Co-op West, Wedgworth
	2	3	1995-1997	Eastgate, Hilliard, Okeelanta Successive
CP 92	1	7	1995-1997	Duda, Knight, Lykes, Okeelanta, Sugar Farms Co-op East, Sugar Farms Co-op West, Wedgworth
	2	3	1996-1999	Eastgate, Hilliard, Okeelanta Successive

selection or model comparison, it was used here as a reflection of the comparative ability of different experimental inputs to fit the physical experimental design as it would occur in a field testing situation. These last two statistics are not of primary interest in plant breeding; however, other agricultural researchers and decision makers may prefer either of the last two statistics. Taken together, these four statistical metrics give insight into the experimental testing design, each from a different perspective and giving slightly different information.

The data were subset and reanalyzed with only two replications from the CP 89 and CP 90 series, two rows per replication, and with only four replications from the CP 91 and CP 92 series to compare results from reduced replication. The first occurring sequential replications were used in each of the four CP series. This subset selection corresponds to the actual physical layout that would be used in the field with reduced replication. Resampling technique, taking all combinations of subsets of four replications, was not used. Such methodology would combine non-contiguously placed replications, and could thus lead to misleading variance components for replication and associated interactions. Repeating the calculations over four CP series, each with two planting sequences, was considered to constitute sufficient resampling of the values with reduced replication, while retaining a tighter correspondence to the physical reality of the experimental design's layout.

Finally, Pearson correlation coefficients and Spearman rank correlation coefficients were formed for each genotype of each CP series among crop years (plant cane, first and second ratoon) and both planting sequences. This was done as an initial way to look into stability of genotypic performance over crop years and planting sequences to evaluate the necessity of testing in three crop years.

# **RESULTS AND DISCUSSION**

The presentation of variance components for each source of variation for all series would be too volumi-

nous. Means and standard errors of all variance component estimates averaged by series and planting sequence are presented in Table 2 by trait. The variance component attributable to crop  $\times$  location interaction was nearly always the largest relative source of variation next to the residual for the first planting sequence. Variation attributable to crop  $\times$  location was high for the second planting sequence, though the variation due to location was also high for all traits. This is not unexpected, as the three locations on which the second sequence is usually planted are quite extreme because of different soil types and planting times.

It should be made clear that there is confounding of year effect with crop effect, as the expediency of the cultivar release program does not allow for planting the same series for more than one year at the same location. This would allow separation of the year and crop effects, the resolution of year  $\times$  crop  $\times$  location interaction, and a year  $\times$  crop  $\times$  genotype interaction. However, Milligan et al. (1990) demonstrated these higher order interactions contribute little to overall variance, and were often difficult to interpret.

The median percent contribution to total variance of each component and its ranges, formed for the four series by planting sequence, are given in Table 3. The median was used rather than the mean due to the distributional properties of these percentages. The median was clearly a more accurate way to summarize than the arithmetic mean formed on raw or transformed data (results not shown).

As would be expected from results in Table 2, the percentage of variation attributable to  $crop \times location$  interaction was nearly always the largest relative source of variation next to the residual for all traits analyzed

Table 2. Means and standard errors of variance components for five traits of replicated sugarcane selection trials, CP 89–CP 92 series, eight replications, by planting sequence.

	First planting sequence										
	Stalk number		Stall	Stalk weight		Cane yield		Theor. rec. sugar		Sugar yield	
Variance Component	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error	
	— No. per plot <sup>2</sup> —		— kg per stalk <sup>2</sup> —		—— Mg ha <sup>-12</sup> ——		kg sugar Mg <sup>-1</sup> cane <sup>2</sup>		—— Mg ha <sup>-12</sup> ——		
$\begin{array}{l} \sigma_G^2 \\ \sigma_C^2 \\ \sigma_{G \times C}^2 \end{array}$	1132.88	604.51	0.0464	0.0294	98.84	53.91	8.04	4.98	0.95	0.61	
$\sigma_{\rm C}^2$	613.19	1098.87	0.1633	0.1841	293.82	356.43	23.23	39.03	7.06	7.89	
$\sigma_{G \times C}^2$	600.17	197.93	0.0228	0.0073	35.76	16.42	5.10	2.23	0.64	0.29	
$\sigma^2$	1462.03	1346.60	0.0646	0.0677	452.47	346.49	18.95	25.74	4.25	3.69	
$\sigma^{L}_{R(L)}$ $\sigma^{2}_{G \times L}$	200.10	56.25	0.0051	0.0019	35.15	10.64	2.20	0.98	0.54	0.17	
$\sigma_{G \times L}^{2}$	354.26	102.97	0.0176	0.0053	54.36	17.70	6.37	2,27	0.82	0.28	
$\sigma_{\mathrm{C} \times \mathrm{L}}^2$	1751.70	662.08	0.1345	0.0544	419.57	172.74	61.05	22,94	5.61	2.32	
$\sigma_{G \times C \times L}^2$	444.04	74.17	0.0160	0.0041	69.35	15.23	5.74	2.08	1.10	0.25	
$\sigma^2_{\text{Row}\{G[R(L)]\}}$	226.03	35.17	0.0000	-	21.57	8.56	1.09	1.72	0.35	0.15	
$\sigma_{Row\{G[R(L)]\}}^2$ $\sigma_{\epsilon}^2$	1315.21	48.02	0.1432	0.0048	434.54	15.82	88.60	3.23	7.32	0.27	
	Second planting sequence										
$\begin{array}{c} \sigma_G^2 \\ \sigma_C^2 \\ \sigma_{\tilde{G} \times C}^2 \end{array}$	1103.78	675.69	0.0628	0.0325	60.20	63.87	16.21	9.98	0.59	0.70	
$\sigma_{\rm C}^2$	710.63	1302.62	0.2798	0.5184	35.51	166.76	12.96	27.94	0.70	2.56	
$\sigma^2_{G \times C}$	350.16	214.11	0.0023	0.0060	42.98	28.64	2.45	10.62	0.63	0.47	
$\sigma_{\rm L}^2$	2498.31	3371.16	0.2663	0.3382	1797.77	2135.70	38.04	49.57	22.56	29.64	
$\sigma_R^2\dagger$	339.84	314.10	0.0007	0.0015	42.66	39.00	0.00	_	0.62	0.58	
$\sigma^2_{R(L)}$ $\sigma^2_{G \times L}$	479.08	169.12	0.0150	0.0070	117.67	45.31	2.16	1.72	1.59	0.88	
$\sigma^2_{G \times L}$	837.46	342.79	0.0252	0.0179	178.95	73.18	7.56	5.64	2.92	1.33	
$\sigma_{C \times L}^2$	1279.07	1287.75	0.1734	0.1258	671.89	518.45	20.27	17.45	8.22	7.49	
$\sigma_{G \times C \times L}^2$	438.09	144.82	0.0147	0.0122	50.76	40.68	1.65	4.15	0.78	0.52	
$\sigma_{\text{Row}[G(R)]}^2\dagger$	694.49	173.78	0.0000	_	62.80	20.26	3.61	12.72	1.13	0.37	
$\sigma_{\text{Row}\{G[R(L)]\}}^{2}$ $\sigma_{\epsilon}^{2}$	486.58	77.77	0.0000	_	23.75	22.44	0.00	_	3.46	_	
$\sigma_{\epsilon}^2$	1302.85	96.19	0.1858	0.0116	540.47	35.84	126.24	9.65	8.85	0.64	

<sup>†</sup> Components from second planting sequence of CP 89, planted at one location only.

<sup>‡</sup> One variance component only, from CP 90 series.

Table 3. Medians and ranges of percent contributions to variance from all model sources for selection trials of four CP sugarcane series (89-92) with eight replications.

	First planting sequence											
	Stalk number		Stalk weight		Cane yield		Theor. rec. sugar		Sugar yield			
Variance Component	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range		
$\begin{array}{l} \sigma_G^2 \\ \sigma_C^2 \\ \sigma_C^2 \times c \\ \sigma_L^2 \times c \\ \sigma_L^2 \\ \sigma_{G(L)}^2 \\ \sigma_{G(X)}^2 \\ \sigma_{G(X)}^2 \end{array}$	14.00	22.82	10.11	12.66	3.33	9.22	4.09	5.34	2.31	5.67		
$\sigma_{\rm C}^2$	6.23	18.47	23.93	43.61	9.19	31.13	<b>5.57</b>	23.69	10.63	41.00		
$\sigma_{G \times C}^2$	4.62	13.10	1.33	9.05	1.41	2.81	1.65	3.30	1.94	4.55		
$\sigma_L^2$	10.64	43.69	13.55	16.71	21.88	21.75	10.07	12.93	13.35	15.92		
$\sigma_{R(I)}^2$	1.51	6.17	0.73	1.19	1.16	3.94	0.85	2.23	1.07	4.22		
$\sigma_{G \times L}^{2(-)}$	5.64	8.74	3.50	7.16	3.21	4.95	3.17	7.48	3.75	5.72		
$\sigma_{C \times L}^2$	25.49	25.75	23.27	17.92	23.26	21.30	27.65	14.35	20.74	13.77		
$\sigma^2_{G \times C \times L}$	4.72	6.40	2.88	3.09	2.96	5.12	2.80	2.49	4.42	6.43		
$\sigma^2_{\text{Row}\{G[R(L)]\}}$	2.52	1.49	0.04	0.09	1.51	1.10	0.61	0.49	2.08	2.40		
$\sigma_{\epsilon}^{2}$	21.13	15.67	24.44	15.56	24.27	17.99	40.91	27.46	30.95	21.25		
	Second planting sequence											
$\begin{array}{l} \sigma_G^2 \\ \sigma_C^2 \\ \sigma_C^2 \times c \\ \sigma_L^2 \\ \sigma_R^2 \dagger \\ \sigma_{R(L)}^2 \\ \sigma_{G\times L}^2 \end{array}$	15.24	20.63	6.84	36.06	3.42	7.82	8.30	14.26	2.29	10.55		
$\sigma_{\rm C}^2$	9.90	15.15	19.05	46.26	1.46	8.32	7.66	14.60	2.15	9.31		
$\sigma_{G \times C}^2$	1.29	21.09	0.42	1.98	1.16	23.07	0.00	3.62	0.80	19.72		
$\sigma_{\rm L}^2$	26.27	11.85	25.38	16.17	49.18	9.19	6.54	34.79	44.81	1.12		
$\sigma_R^2\dagger$	0.00	_	0.37	-	0.08	_	0.00	_	0.00	_		
$\sigma_{R(L)}^2$	1.49	6.76	1.33	0.13	2.01	4.36	0.13	1.96	2.70	5.60		
$\sigma^2_{G \times L}$	3.63	31.14	13.80	43.17	19.18	12.17	8.70	10.94	14.87	11.44		
$\sigma_{c \times L}$	9.69	12.99	1.70	4.14	4.07	7.70	4.92	5.55	5.81	10.65		
$\sigma_{G \times C \times L}^2$	5.13	4.38	1.07	4.31	0.61	5.26	0.00	1.82	0.45	6.06		
$\sigma_{\text{Row}[G(R)]}^{z}$	14.92	_	0.00	_	19.39	_	0.00	_	16.72	_		
$\sigma_{\text{Row}[G[R(L)]]}^2$ ; $\sigma_{\epsilon}^2$	9.63	_	0.00	_	3.13	_	0.84	_	2.85	_		
$\sigma_{\epsilon}^2$	14.62	13.25	23.60	31.77	19.60	23.74	58.62	30.43	25.46	21.67		

<sup>†</sup> Components from second planting sequence of CP 89, planted at one location only.

Table 4. Medians and ranges of percent contributions of sources of variance for selection trials for CP sugarcane series (89-92) with four replications.

	First planting sequence											
	Stalk number		Stalk weight		Cane yield		Theor. rec. sugar		Sugar yield			
Variance Component	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range		
$\sigma_G^2$	15.44	23.88	10.63	11.83	3.29	10.29	3.73	6.34	2.07	7.15		
$\sigma_c^2$	8.29	15.22	22.53	46.20	10.84	32.91	5.45	24.69	11.47	43.39		
$\begin{array}{c} \sigma_G^2 \times c \\ \sigma_L^2 \\ \sigma_{R(L)}^2 \end{array}$	4.64	13.86	1.71	8.35	1.44	4.24	1.32	4.62	1.80	6.05		
$\sigma_{\rm L}^2$	12.01	47.12	8.99	15.94	25.04	19.23	10.43	14.78	16.16	16.31		
$\sigma_{R(I)}^2$	1.40	4.89	1.04	1.50	0.96	2.31	0.45	3.53	1.14	2.87		
$\sigma_{c} \times 1$	5.28	9.04	4.92	7.47	3.87	5.69	4.18	4.29	3.87	6.46		
$\sigma_{\text{C}}^2 \times L$	22.25	21.23	24.30	20.59	23.33	15.71	28.95	16.17	22.53	14.17		
$\sigma_{G \times C \times L}^2$	3.42	9.41	4.07	3.62	2.72	7.42	2.20	3.64	3.30	10.58		
$\sigma_{\text{Row}\{G[R(L)]\}}^2$	2.46	1.34	0.00	0.00	1.25	0.98	0.30	0.07	1.35	1.20		
$\sigma_{\epsilon}^{2}$	17.81	18.23	24.12	14.86	23.34	16.22	41.71	30.40	28.65	22.07		
	Second planting sequence											
$\begin{array}{l} \sigma_G^2 \\ \sigma_C^2 \\ \sigma_G^2 \times c \end{array}$	14.84	13.17	4.63	37.41	1.56	6.32	4.35	11.87	3.06	6.07		
$\sigma_{\rm C}^2$	10.32	19.09	26.22	52.84	3.98	8.51	3.23	15.49	4.55	10.72		
$\sigma_{G \times C}^2$	1.31	19.08	0.72	2.81	1.61	20.04	0.00	0.35	0.74	13.99		
$\sigma_{\rm L}^2$ $\sigma_{\rm R}^2 \dagger$ $\sigma_{\rm R(L)}^2$	35.52	19.67	17.81	15.54	40.87	15.84	10.81	43.37	40.15	16.26		
$\sigma_{R}^{2}\dagger$	7.39	_	0.63	_	11.57	_	0.60	_	7.84	_		
$\sigma_{R(L)}^2$	2.43	1.29	1.08	1.04	2.62	3.39	0.00	0.00	3.08	4.26		
$\sigma_{G \times L}^{2}$	5.43	13.34	2.99	4.21	3.05	6.02	5.03	5.33	6.01	6.31		
$\sigma_{\mathrm{C} \times \mathrm{L}}^{2}$	4.26	38.69	19.18	46.57	26.13	24.28	9.87	10.29	19.60	25.46		
$\sigma_{G \times C \times L}^2$	6.49	4.77	1.30	4.21	1.32	7.17	0.00	0.00	1.31	10.90		
$\sigma_{\text{Row}[G(R)]}^2\dagger$	11.82	_	0.00	_	11.40	_	0.00	_	10.82	_		
σ <sup>2</sup> <sub>Row(G(R(L)))</sub> ‡	7.29	_	0.00	_	1.45	_	0.00	_	0.65	_		
$\sigma_{\text{Row}[G[R(L)]]}^2$ ;	17.12	20.35	23.47	29.34	18.18	34.00	58.05	48.83	25.22	32.66		

<sup>†</sup> Components from second planting sequence of CP 89, planted at one location only. ‡ One variance component only, from CP 90 series.

in the first planting sequence. Graphical three-dimensional plots of crop and location means on the x- and y-axes and each response variable the z-axis (not shown) were used to investigate the nature of this interaction. The majority of this interaction was due to irregular, but generally constant, declines in sugar yield and its components, as trials aged from the plant crop to the

second-ratoon crop. Rank changes of crops at certain locations also contributed to this interaction in a minority of instances, mostly due to known, specific local environmental effects characteristic of certain locations, such as drought, winter freezes, etc. The percentage of variation attributable to this source was generally large for the second planting sequence also, though again, the

<sup>‡</sup> One variance component only, from CP 90 series.

Table 5. Comparisons of four statistical metrics from four CP-series Stage IV trials, with eight replications and four replications: (1) experiment-wise coefficient of variance (%CV), (2) genetic repeatability (unitless), (3) genetic coefficient of variance (%GCV), and (4) R<sup>2</sup> of model fit to data using full model.

CP Series 1989					Eight replic	ations	Four replications				
	Planting seq.	Trait	Locations	%CV	Genetic repeatability	%GCV	$R^2$	%CV	Genetic repeatability	%GCV	$R^2$
	1	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	9 (8-R1†)	11.32 13.29 17.37 7.29 19.55	0.40 0.00 0.76 0.46 0.72	6.75 0.00 7.25 1.68 7.03	0.94 0.89 0.90 0.82 0.89	10.70 12.30 16.49 7.04 18.68	0.40 0.00 0.79 0.44 0.68	6.71 0.00 6.79 1.63 1.90	0.95 0.91 0.92 0.85 0.91
	2	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	1	18.07 12.30 22.11 11.51 24.37	0.98 0.88 0.83 0.89 0.24	18.37 11.07 9.27 5.04 11.48	0.86 0.69 0.76 0.55 0.75	21.10 11.13 25.29 13.44 29.98	0.93 0.95 0.29 0.00 0.20	16.19 10.67 3.75 4.96 3.04	0.85 0.73 0.74 0.55 0.72
1990	1	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	7	10.60 12.70 16.65 6.56 18.48	0.84 0.87 0.63 0.81 0.36	13.61 7.84 7.93 3.28 5.03	0.93 0.84 0.88 0.80 0.84	10.07 12.44 16.40 5.52 17.65	0.85 0.85 0.58 0.82 0.29	13.81 7.81 7.60 3.06 4.64	0.94 0.86 0.89 0.86 0.86
	2	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	3	9.65 13.87 18.60 8.67 18.30	0.89 0.87 0.73 0.92 0.77	15.72 7.68 10.07 3.49 9.45	0.96 0.88 0.91 0.77 0.90	8.18 14.09 16.57 9.67 20.51	0.88 0.60 0.67 0.65 0.65	14.79 5.97 11.56 2.55 11.50	0.98 0.89 0.95 0.82 0.93
1991	1	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	7	13.81 13.17 18.66 9.45 21.01	0.77 0.91 0.53 0.65 0.57	7.45 8.56 4.65 1.79 5.70	0.75 0.72 0.71 0.51 0.67	14.09 12.41 17.94 9.33 20.27	0.78 0.83 0.56 0.49 0.54	8.14 7.85 5.34 1.46 5.52	0.79 0.78 0.78 0.58 0.75
	2	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	3 (2-R2†)	14.59 15.43 21.58 9.18 23.93	0.50 0.78 0.15 0.12 0.00	8.43 8.59 3.94 0.89 0.00	0.79 0.79 0.80 0.54 0.74	13.49 13.54 20.46 8.68 22.86	0.55 0.67 0.00 0.00 0.00	8.66 7.80 0.00 0.00 0.00	0.84 0.87 0.85 0.66 0.78
1992	1	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yield	7 (6-PC†)	13.18 13.46 17.84 8.55 20.01	0.92 0.94 0.88 0.73 0.81	14.75 8.98 10.98 2.99 9.12	0.79 0.75 0.73 0.55 0.67	14.50 12.82 17.76 8.81 19.96	0.92 0.89 0.88 0.73 0.82	15.49 8.47 11.63 2.89 9.76	0.80 0.79 0.77 0.58 0.72
	2‡	Stalk no. Stalk wt. Cane yld. Theor. rec. sugar Sugar yld.	3	14.49 15.05 21.24 7.24 22.65	0.59 0.78 0.37 0.68 0.00	9.52 7.69 5.38 2.44 0.00	0.85 0.80 0.82 0.51 0.78	13.46 14.40 21.42 7.55 23.30	0.81 0.50 0.48 0.47 0.10	12.95 5.71 7.09 2.18 2.93	0.87 0.83 0.83 0.60 0.77

<sup>† (8-</sup>R1) denotes 8 locations in first ratoon crop, (2-R2) 2 locations in second ratoon, and (6-PC) 6 locations in plant cane crop.

percentage of variation due to location effect was also generally higher, due to the extremeness of these locations, as previously explained. The variance contribution due to genotype  $\times$  crop interaction was rather low for the first planting sequence, as was genotype  $\times$  location interaction. Nonetheless, these two interactions cannot be discounted in genotype (cultivar or clonal) release decisions.

The small relative magnitude of variance contributed by replications strongly indicates that maintaining eight replications, or four replications with two subsampled rows, is excessive. Median percent contributions to total variance of each source of variation based on four replications, rather than eight (or two replications with two subsamples) are presented for each trait by planting sequence in Table 4. The relative percent contribution to variance from replications does not generally increase, and in fact actually decreases for four out of the five traits for the first planting sequence. Table 5 contains the %CV, genetic repeatability, GCV, and  $R^2$  values generated from eight replications versus four rep-

lications. Again, there is very little difference in magnitude of these values by planting half the usual number of replications. The choice of four replications was not arbitrary. Replications constitute an extremely low source of variability (Table 3), indicating that the number of replications could be reduced substantially. Milligan (1994) stated that the Louisiana Sugarcane Variety Development Program is carried out with only three replications per location. We propose lowering the number of replications to four, a dramatic drop from previous effort. If one replication were to be lost, three replications would still remain, which is considered sufficient in the Louisiana Sugarcane Variety Development Program, and which our numbers also indicate should be sufficient.

The number of locations affects the calculation of genetic repeatability as well as the error term for mean separation among genotypes using a mixed model approach, as would normally be done in analyzing these trial results. Number of locations, therefore, affects the statistical precision of the data used to make cultivar

<sup>‡</sup> Plant cane and first ratoon crops only.

decisions. In addition, the inference space is larger for the assumption that the set of locations constitutes a random sample of environments in which the genotypes would be grown commercially. Results from analyses separating planting sequences in Tables 2, 3, and 4 also suggest that the second planting sequence mirrors the results of the first planting sequence, though with less precision. The data cannot be combined into one model across planting sequences because the location effect would be inflated by the consequent confounding with different year effects, as the locations are different in the second planting sequence and are planted nearly a year later. Interpretation of results from the second sequence is also difficult due to the comparison of genotype performance in different locations planted in different years. The elimination of the second planting sequence with concurrent expansion of the number of locations, genotypes, and testing conditions in the first planting sequence would be a beneficial change of action.

It was of interest to know whether it was necessary to test through the second-ratoon crop. As both the Pearson and Spearman rank correlation coefficients for each genotype over crops (not shown) tended to decline from the plant-cane crop to the first-ration crop to the second-ratoon crop, it was deemed necessary to continue to grow all three crops. There were a few exceptions to this trend of declining correlations, with some clones showing higher coefficients with increasing crop age; however, as variable decline of yield with increased crop age was the rule, these correlation coefficients showed a trend downward in the majority of cultivars. Moreover, variance components for crop and for interaction components involving crop were relatively large, further indicating the need for testing all three crops. The fact that most of the sugarcane crop in the Everglades Agricultural Area is grown to the second ratoon, together with the above results, shows the need to continue to test all three crop years. Some growers continue even beyond the second ratoon; however, the area grown beyond second ratoon represents only about 10% of the total crop in Florida. Therefore, it was not deemed appropriate to redirect the allocation of resources in this direction.

As mentioned previously, the scientists who conduct this sugarcane selection program need to redirect resources to include selection of genotypes with positive environmental impact. Our results show that reducing replications within locations from eight to four and reducing planting sequences from two to one would allow

us to continue making decisions about Stage IV clones at the current level of statistical precision. Redirecting the resources gained by making these changes would allow screening for traits such as water-table tolerance and P uptake. Thus, we could begin selection for environmentally related characters in Stage IV trials and maintain or increase our current level of effort for standard agronomic and pathological characters without increasing input resources.

### **ACKNOWLEDGMENTS**

We wish to acknowledge the valuable assistance of Eric Stafne in preparation and proofing of this manuscript, as well as the helpful reviews and suggestions of Dr. Scott Milligan and Jim Shine.

### REFERENCES

- Deren, C.W., S.H. Snyder, J.D. Miller, and P.S. Porter. 1991. Screening for and heritability of flood-tolerance in the Florida (CP) sugarcane breeding population. Euphytica 56:155–160.
- Glaz, B., J.M. Shine, Jr., P.Y.P. Tai, J.D. Miller, C.W. Deren, J.C. Comstock, and O. Sosa, Jr. 1994. Evaluation of new Canal Point sugarcane clones: 1993-94 harvest season. USDA/ARS, ARS-109-1993. U.S. Gov. Print. Office, Washington, DC.
- Glaz, B., P.Y.P. Tai, J.D. Miller, C.W. Deren, J.M. Shine, Jr., J.C. Comstock, and O. Sosa, Jr. 1995. Evaluation of new Canal Point sugarcane clones: 1994-95 harvest season. USDA/ARS, ARS-109-1994. U.S. Gov. Print. Office, Washington, DC.
- Glaz, B., C.W. Deren, and G.H. Snyder. 1997a. Variability of leaf phosphorus among sugarcane genotypes grown on Everglades histosols. J. Environ. Qual. 26:1707-1711.
- Glaz, B., J.D. Miller, C.W. Deren, P.Y.P. Tai, J.C. Comstock, and O. Sosa, Jr. 1997b. Evaluation of new Canal Point sugarcane clones: 1995-96 harvest season. USDA/ARS, ARS-140. U.S. Gov. Print. Office, Washington, DC.
- Glaz, B., P.Y.P. Tai, J.C. Comstock, and J.D. Miller. 1998. Evaluation of new Canal Point sugarcane clones: 1996-97 harvest season. USDA/ARS, ARS-146. U.S. Gov. Print. Office, Washington, DC.
- Kang, M.S., G.H. Snyder, and J.D. Miller. 1986. Evaluation of Saccharum and related germplasm for tolerance to high water table on organic soil. J. Am. Soc. Sugarcane Technnol. 6:59-63.
- Milligan, S.B., K.A. Gravois, K.P. Bischoff, and F.A. Martin. 1990. Crop effects on broad-sense heritabilities and genetic variances of sugarcane yield components. Crop Sci. 30:344-349.
- Milligan, S.B. 1994. Test site allocation within and among stages of a sugarcane breeding program. Crop Sci. 34:1184-1190.
- SAS Institute Inc. 1997. SAS/Stat software: Changes and enhance-
- ments through Release 6.12. SAS Inst. Inc., Cary, NC. Stone, J.A., and D.E. Legg. 1992. Agriculture and the Everglades. J. Soil Water Conserv. 47:207-215.
- Tai, P.Y.P., and J.D. Miller. 1989. Family performance at early stages of selection and frequency of superior clones from crosses among Canal Point cultivars of sugarcane. J. Am. Soc. Sugarcane Technol. 9:62-70.
- Whalen, B.M., and P.J. Whalen. 1994. Nonpoint source regulatory program for the Everglades Agricultural Area. Paper FL94-101. Florida Section of the American Society of Agricultural Engineers.